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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Preparation of m(co)3-complexes by solid phase techniques via metal assisted cleavage from the solid support

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PREPARATION OF $M(CO)_3$-COMPLEXES BY SOLID PHASE TECHNIQUES VIA METAL ASSISTED CLEAVAGE FROM THE SOLID SUPPORT

The invention relates to the field of radiopharmaceuticals. In particular the invention relates to a process for the preparation of a metal complexed agent via metal assisted cleavage from a solid support.

In a further aspect the invention relates to new solid phase bound conjugates of a ligand and a biomolecule.

In yet a further aspect the invention relates to new metal complexed ligand-biomolecule conjugates, compositions comprising these new complexes and their use.

In still a further aspect the invention relates to a kit for the preparation of a diagnostic or therapeutic pharmaceutical composition.

For the application of radiolabeled bioactive molecules such as i.e. peptides in clinical routine diagnosis or therapy it is highly desirable that only labeled compounds are injected to avoid saturation of the corresponding receptors *in vivo* or toxic side effects from "cold", unlabeled compounds. Furthermore, binding of large amounts of unlabeled biomolecules to the receptors spoils the possibility of getting clear images (scintigrams) and, thus, often disables a clear diagnosis.

According to the state of the art, high specific activity in a normal homogenous labeling procedure can only be achieved by using the lowest possible amount (concentration) of derivatized biomolecules (or ligand for ^{99m}Tc which is coupled to the biomolecule) which still results in quantitative labeling. Depending on the ligand and the complex precursor, these amounts often have to be relatively high since at low concentrations the rate of complexation is governed by a second

order kinetic and, thus, labeling is too slow and accompanied by decomposition of ligand or ^{99m}Tc precursor. The lowest concentration limit is often not convenient in routine use, since slightly changed conditions (temperature, time) at such a concentration do not end up with quantitative labeling yield. Correspondingly, side- and decomposition products as well as starting materials are still present in the final solution.

A convenient way of a physically separating 'cold' from 'hot' compound is by attaching the ligand-biomolecule conjugate to a solid phase material and cleave it from there concomitantly with the complex formation. Examples for such metal assisted cleavage from solid phases are rare.

American patent US-5,789,555 (Pollak et al.) describes a process for labeling peptides with technetium-99m, rhenium-186 or rhenium-188. The process comprising the steps of covalently coupling the peptides to a solid support, by means of a thioether bond with a maleimide linker. By introducing pertechnetate to the support, a $^{99m}Tc^{V}(=O)$-peptide complex is formed. Upon complex formation, $^{99m}Tc^{V}(=O)$ catalyzes cleavage of the peptide from the support, by breaking the C-S bond, thus releasing the $^{99m}Tc^{V}(=O)$-peptide complex from the support.

It is known from literature, that protected thiols release the protecting group by coordination to a Tc=O center. Based on these findings Pollak et. al. (J. Am. Chem. Soc. 121, 11593-11594 (1999) bound a tetradentate chelator via a thioether bond to a gold surface. Upon coordination of Tc(V) to this ligand the ^{99m}Tc-complex was selectively released into solution by breaking the S-Au-bond as the sulfur coordinated to the Tc.

More recently, Dunn-Dufault, et. al. (Nucl. Med. Biol. 27, 803-807 (2000)) described a variant of this method by

covalently binding the chelator to an organic polymeric support.

The above mentioned processes for producing Tc and Re labeled organic complexes all depend on cleavage of a C-S or Au-S bond. This C-S and Au-S bond, with which the ligand is covalently bound to the solid support, is sensitive to oxidation. Therefore, it is necessary to store solid supports comprising ligands covalently linked via a C-S bond under reducing conditions. This is especially true for long term storage. The necessity of storage under reducing conditions requires additional measures to be taken for storage. Moreover, if the supports are to be used for the generation of compounds suitable for pharmaceutical applications, the presence of reducing agents is highly undesirable from the standpoint of pharmaceutical safety. Therefore, there will be certain restrictions for use of the known solid bound ligands for such applications.

Additionally, the use of these metal oxide species is accompanied by restrictions to the ligands that are available for use therewith i.e. tetradentates. Hence the sole disclosure in the prior art of peptidic ligands for use with a $^{99m}Tc^{V}(=O)$ center.

Thus there is a need for new processes for preparing metal labeled complexes by solid phase techniques via metal assisted cleavage from the solid support which employ solid phase bound biomolecule-ligand conjugates which are more stable under pharmaceutically acceptable conditions than the prior art conjugates.

Additionally, the availability of more ligands that can be used in the formation of metal complexed ligand-biomolecule conjugates by means of solid phase techniques via -

metal assisted cleavage will be advantageous, since this will provide a more flexible use of this technique. It is the object of the present invention to provide improved techniques for the preparation of labeled diagnostic and therapeutic compounds.

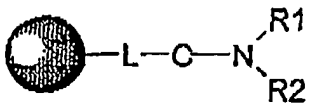
In the research that led to the present invention, it was found that some organic molecules, bound to a solid support via a tridentate ligand (L) that is coupled to the support through its tertiary amino group, in the presence of $[Tc(H_2O)_3(CO)_3]$, cleave from the ligand upon formation of $[Tc(CO)_3$-Ligand]-complexes. The selective hydrolytic C-N bond cleavage is clearly mediated by the low valent carbonyl $[TC(H_2O)(CO)_3]$ center, formed during complex formation, and does not occur under the same reaction conditions in the absence of $[^{99m}Tc(H_2O)_3(CO)_3]$.

The mechanism is proposed to be as follows. As the tertiary amino group of the solid-bound chelator (the so-called ligand) coordinates to the cationic metal center of $M(H_2O)_3(CO)_3$, it becomes partially positive and the adjacent carbon atom is therefore activated for nucleophilic attack. A remaining hydroxy group attacks the methylene group of the chelator and induces C-N bond cleavage (Figure 2). The third donor site of the chelator coordinates to the metal center, and the product complex is released into solution. Uncomplexed chelator and uncleaved complex remain bound to the solid phase.

It was found that labeled compositions obtained by hydrolytic cleavage of the ligand from the solid support with $[^{99m}Tc(H_2O)_3(CO)_3]$ as described above had a high specific activity i.e. there was little uncomplexed ligand in solution. The amount of uncomplexed ligand in solution was in the order of 10^{-7} M. Therefore, this specific cleavage reaction can be attractively exploited for the preparation of so-called "no

carrier added" (n.c.a.) complexes of technetium and other metals with a similar chemical reactivity.

Thus the invention relates to a new process for generating a metal complexed agent, comprising contacting (I) a solid phase bound organic conjugate represented by the formula



(I)

wherein:

C is a methylene group that may be substituted by one or more electron withdrawing groups, in particular RO, RS or R N, wherein R is an aliphatic or arylic group,

L is a linker that may or may not be present, that is coupled to the solid support and has activating properties towards nucleophilic attack to the C group and is preferably a phenyl, allyl or aryl; and

R1 and R2 are the same or different metal coordinating group, which solid phase bound organic conjugate is derivatized at one or both of R1 and R2 with a biologically active molecule,

with (II) $[M(H_2O)_3(CO)_3]$,

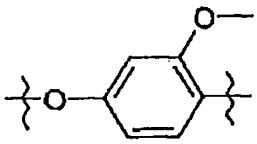
wherein M is selected from the group consisting of technetium (Tc), rhenium (Re), rhodium (Rh), platinum (Pt), iridium (Ir) and copper (Cu);

under suitable conditions to cause the formation of a coordinate bond between $[M(H_2O)_3(CO)_3]$ and the tertiary amine

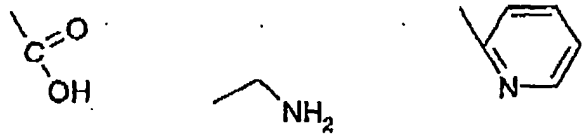
nitrogen atom of the solid phase bound organic conjugate and thereby the release of the metal complexed agent thus formed from the support.

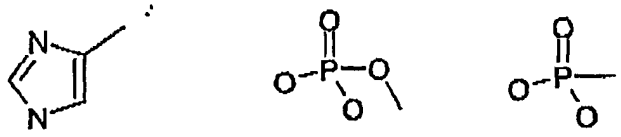
The linker may or may not be present. When it is present it is preferably a good activating group for nucleophilic attack and selected from the group consisting of phenyl, vinyl, aryl and other non-aliphatic groups. The phenyl, vinyl, aryl or other non-aliphatic group may be substituted, and if they are they are preferably substituted with an electron withdrawing group selected from OR, R, NR_2.

In a preferred embodiment the linker is a group of the formula



Preferably, R1 and R2 are selected from the group consisting of





The metal M may be any metal and is preferably selected from the group consisting of Tc, Re, Ru, Rh, Ir, Cu and Pt. The metal is most preferably ^{99m}Tc, ^{186}Re, ^{188}Re.

Preferably the metal is suitable for use as an

imaging agent, e.g. by transmission of high-energy particles or paramagnetic characteristics, or as a radionuclide.

$[M(H_2O)_3(CO)_3]^+$ can be generated by any suitable means known in the art. Suitable means for generating $[M(H_2O)_3(CO)_3]^+$ are for example from the permetallate form as disclosed by Alberto et al. (J. Am. Chem. Soc. **123**, 3135-3136 (2001)) or in WO98/48848 (Alberto et al.).

The molecule according to formula I without the solid support is called herein the ligand, in particular the tridentate ligand or the chelator.

The ligands used according to the invention in combination with $[M(H_2O)_3(CO)_3]^+$ can be a diversity of tridentate ligands, the main requirement being the presence of a tertiary amine group as the central part of the ligand, which forms the C-N bond that is coupled with the solid support and cleaved upon complex formation. Preferably the ligands used are those based on aliphatic or aromatic amines or carboxylates combinations thereof as donors.

In particular diethylene triamine, picolylamine-*N*-acetic acid, or *N*-(2-aminoethyl)-glycine can be used as ligands in the invention.

The ligand can be covalently linked to the solid support by first forming a halogenated resin e.g. by the methods described by Ngu and Patel (Tet. Lett. 38, 973 - 976 (1997)). This halogenated resin can subsequently be reacted with a protected ligand. After deprotection, the ligand bound resin is obtained. If the ligand is attached to the solid phase by this method the covalent bond attaching it to the solid support will be a C-N bond. Preparation of a loaded resin is discussed in more detail in example 1.

In this specification the term ligand refers to a

compound comprising at least one metal coordinating atom capable of forming a coordinating bond with a metal to form a stable metal-ligand complex. A ligand comprising more than one metal coordinating atom may be referred to as a chelator or a multidentate ligand. Tridentate ligands are ligands with three metal-coordinating atoms and tetradentate ligands are ligands with four metal coordinating atoms.

The biologically active molecule (also called herein "biomolecule") that is coupled to the ligand can be any molecule that is active in diagnosis or therapy. It may be a targeting molecule for directing the radioactive product to the site that needs to be diagnosed or treated or it may have a therapeutic activity that is independent from the radiolabel. The biologically active molecule may be selected from the group consisting of amino acids; steroids; peptides; proteins, in particular structural proteins, enzymes or antibodies; carbohydrates; polysaccharides and oligosaccharides; nucleosides, nucleotides, oligonucleotides and polynucleotides; lipids, peptides.

The biomolecule can be linked to the ligand with any suitable means known in the art e.g. by reductive amination of an aldehyde to a primary amine group of the ligand or by introducing a binding site at the arylic system. The biomolecule can be linked to the ligand prior to or after binding the ligand to the solid support.

It was found that the choice of the solid support may further improve the efficiency of the process of the invention. The solid support has to be able to swell in water, it has to be stable at reaction conditions, and it must not contain metal coordinating units. This is in particular the case when the solid support is a polyethylene glycol resin, or a hybrid of

polyethylene glycol and polystyrene, e.g. a polystyrene resin with polyethylene glycol spacers with a benzyl alcohol anchoring group.

The process of the invention may further comprise the step of collecting the metal complexed agent (i.e. the radiopharmaceutical) for further use.

After preparation of n.c.a. ^{99m}Tc radiopharmaceutical, the solid phase polymer can be collected, washed and reused.

Preferably, the process is performed at a pH that is in the range of about 6.0-11.0, preferably in the range of about 7.5-9.5.

Suitable temperatures for performing the reaction are within the range of about 40-100°C. Preferably the reaction is performed in the range of about 70-82°C.

According to a further aspect thereof the invention relates to the solid phase bound ligand-biomolecule conjugate of formula I and compositions comprising such a compound. Preferably these compositions are in a form which can be stored during prolonged time periods under pharmaceutically acceptable conditions.

With the process according to the invention metal complexed ligand-biomolecule conjugates can be obtained with a high specific activity by filtration without further post-labeling purification.

According to a further aspect thereof the invention relates to the metal complexed ligand-biomolecule conjugates obtainable with the process according to the invention. Usually the conjugates are comprised in a composition which is the result of the process of the invention, and which is characterized in that it is essentially free of uncomplexed ligand-biomolecule conjugate e.g. the level of uncomplexed

ligand-biomolecule conjugate in the composition containing them is in the 10^{-7} M range. Compositions with such characteristics are a further aspect of the invention.

Due to the short half-life of some isotopes used in radiopharmaceuticals, e.g. ^{99m}Tc, labeling of ligand-biomolecule conjugates just prior to their use can be important for the specific activity of the complexed conjugate. The amount of decayed complex in a freshly labeled composition will be lower compared to the situation when the conjugate was complexed a substantial amount of time prior to its use.

Therefore, in yet a further aspect the invention relates to a kit for the preparation of a diagnostic or therapeutic pharmaceutical composition, comprising a container with the molecule of formula (I), in which the reaction with a solution of $[M(H_2O)_3(CO)_3]$ can take place. The container can be a vessel or column. The solution of $[M(H_2O)_3(CO)_3]$ is introduced into the vessel or column to start the reaction. The solution can be part of the kit or provided by other means. In an alternative embodiment, the reagents for the preparation of the metal carbonyl $[M(H_2O)_3(CO)_3]$ are comprised in the kit. In addition, the kit may comprise a facility for filtration.

The use of a kit further provides flexibility to the metal complex that can be formed since a selection of a suitable metal can be made just prior to the complexation reaction.

The principle of the preparation of no carrier added (n.c.a.) metal complexed compounds according to the invention is explained in figure 1. A tridentate ligand e.g. diethylene triamine is bound via a linker, here a benzyl derivative, to a solid phase via a tertiary amine. To the chelator (ligand) a biomolecule, is attached, thus forming a ligand-biomolecule

conjugate. Upon introduction of $[Tc(H_2O)_3(CO)_3]$, complex formation occurs and the tridentate ligand replaces the two aqua ligands. The remaining hydroxy ligand on $[Tc(H_2O)_2(OH)(CO)_3]$ can now attack the activated methylene group to induce C-N-bond cleavage. Activation of the methylene group occurs by complexation of the tertiary amino group to the technetium center which withdraws electron density from the chelator.

The main species with reactivity towards the tertiary amine atom of the solid phase bound biomolecule-ligand conjugate is $[M(OH)(H_2O)_2(CO)_3]$. In solution $[M(OH)(H_2O)_2(CO)_3]$ is in equilibrium with the undissociated form $[M(H_2O)_3(CO)_3]$ and further dissociated forms, depending on the pH of the solution. It will be understood that, depending on the pH, $[M(OH)(H_2O)_2(CO)_3]$ is at least partially interchangeable with these species due to the equilibrium.

The invention is further explained with the following non-restrictive examples.

EXAMPLES

EXAMPLE 1

Two model ligands were covalently attached to an appropriate solid phase resin. The solid phase resin has to swell in water to allow diffusion of the Tc-species, it has to be stable up to 90°C, and the anchoring group to which the ligand is coupled has to be an activating group for nucleophilic attack.

The polystyrene/polyethylene glycol resin TentaGel S AC (Rapp Polymere GmbH, Tübingen, Germany) fulfills these requirements. Its active site, a benzyl alcohol derivative, was converted into the corresponding bromide1 (Ngu and Patel, Tet.

Lett. 38, 973 - 976 (1997)). This compound reacted with $N^{1,5}$-bis(1-(4,4-dimethyl-2,6-dioxocyclohexyliden)ethyl)-1,5-diamino-3-azapentane (2) to give, after deprotecting with hydrazine hydrate, solid-phase bound chelator 3 (Figure 3a). Chelator 4 was synthesized by reacting 1 with *N*-picolylamine acetic acid ethylester (5) and successive basic hydrolysis of the ester group (Figure 3b). Resin 6 was prepared by a similar procedure.

The reactions on the solid phase were followed by coloring reactions on sample beads. A solution of bromophenol blue in DMF colored the basic compounds 3, its protected intermediate and the protected intermediate of 4, but not the zwitterionic product 4. Product 3 was the only compound which could be colored with trinitrobenzoesulfonic acid (TNBS), a regent with selectivity for primary amines. The nitrogen content of compounds 3 and 4 was determined by elementary analysis and could be used for calculating the capacity of the resins, i.e. the mmoles of chelating units per gram of solid phase material. Values were 0.24 mmole/g for 3 and 0.21 mmole/g for 4, corresponding to coupling efficiency of 100% and 88%, respectively.

The chelating capacity of resin 3 was verified by stirring it in a 1 mM solution of $[^{99}Tc(H_2O)_3(CO)_3]^+$ (7 equivalents) at room temperature. Analyzing of the filtrated solution by β^- liquid scintillation counting showed a decrease of activity of 14% which is consistent with quantitative complex formation on the resin. HPLC analyses exhibited only peaks of the starting material, indicating that no cleavage from the solid phase occurred under these mild conditions. The once formed ^{99}Tc-complex turned out to be stable under the conditions used for labeling. Even prolonged heating at 80°C for 5 hours in phosphate buffer pH 7.5, yielded only 3% β-

activity in the solution, at least one order of magnitude lower than expected.

EXAMPLE 2

Resin 7 (table 1) was synthesized on the solid support. *N*-Boc-*N''*-Dde protected diethylene triamine was coupled to the brominated TentaGel S AC resin, the Dde protecting group was removed, and the pyrene group was introduced by reductive amination using 1-pyrenaldehyde and sodium triacetoxyborohydride. Finally, the Boc protecting group was removed with trifluoroacetic acid (50% in CH_2Cl_2).

EXAMPLE 3

Labeling conditions: $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared out of $[^{99m}TcO_4]^-$ using a borocarbonate kit (Alberto et al, *J.Am. Chem. Soc.* 123, 3135-3136 (2001)). 1 mg of the solid-phase bound chelators (0.2 mmole) were given to the $[^{99m}Tc(H_2O)_3(CO)_3]^+$-solution (1 ml), the mixtures were shortly sonificated and then heated to 82°C for 30 minutes. The solutions were separated from the solid phase resin by filtration and analysed by HPLC with γ-detection.

With all of the solid phase bound chelators, formation of soluble complexes was observed. The yield varied on chelator type and reaction conditions between 5 to 50% (Table 1).

EXAMPLE 4

Resins 3 and 4 were also labeled in a one pot procedure, combining the formation of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and the cleavage reaction. 1 mg of solid-phase bound chelators (0.2 mmole) were added to a borocarbonate kit (Mallinckrodt Medical,

Petten, the Netherlands; Alberto et al., J. Am. Chem. Soc. 123, 3135-3136 (2001)). $NaTcO_4$ as eluted from a generator was added to the vial, and the mixture was kept at 78°C to 82°C for 20 to 60 minutes. The pH was 11. Cleavage yield was between 8 and 32%, conversion of pertechnetate between 40 and 54%.

EXAMPLE 5

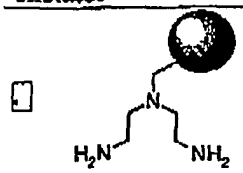
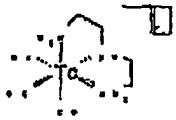
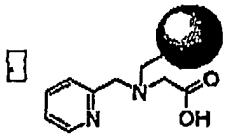
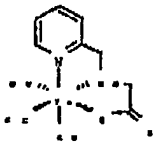
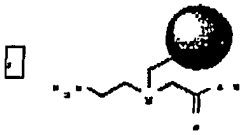
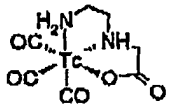
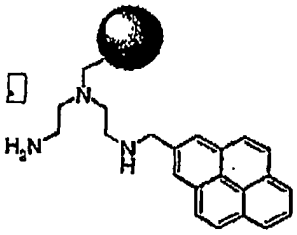
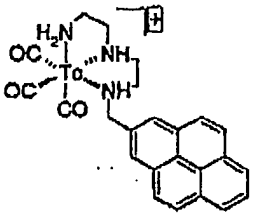
Reaction conditions such as pH value and reaction temperature were varied to find optimal reaction conditions. For use as a radiotracer, complete conversion of the starting material $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and formation of one single product is required. Purification steps after the labeling procedure have to be avoided because of the radioactivity of the samples and their rapid decay ($t_{1/2}$ = 6.2 hours). High cleavage yields are desirable to get solutions of high radioactivity.

pH dependence of the cleavage reaction is shown in Figure 4. Cleavage yield increases from pH 6 with a maximum at pH 8.5. This is in consistence with deprotonation of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ to $[^{99m}Tc(OH)(H_2O)_2(CO)_3]$ (pK_s for the Rhenium analog: 7.5; Egli et al, *Organometallics* 16, 1833-1840 (1997)) and, therefore, with the theory that a Tc-coordinated hydroxy ion is the nucleophile which attacks the CH_2-group to cleave the C-N bond. Increasing the pH to 11 reduces the cleavage yield again. This could be due to formation of the negatively charged species $[^{99m}Tc(OH)_2(H_2O)(CO)_3]^-$ which reduces the electrophilicity of a coordinated amino group.

Temperature dependence of the reaction of resin 4 with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was studied by analyzing the reaction products after full conversion of $[^{99m}Tc(H_2O)_3(CO)_3]^+$. At room temperature, only complex formation at the resin occurred, the solution after filtration from the solid phase exhibited no

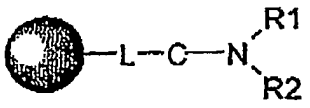
radioactivity. At 43°C, cleavage yield was observable but low. Then, it increased with increasing temperature (Figure 5). This clearly shows that there is a competition between complex formation at the resin and the cleavage reaction, with the cleavage reaction having the higher activation energy barrier. However, very high temperatures could be a disadvantage in view of the stability of the solid phase resin and of attached biomolecules. A reaction temperature of 70°C to 82°C is preferred.

Table 1

Solid-phase bound chelator	cleaved ^{99m}Tc-complex	cleavage yield	Remarks
		13 %, + 14 % intermediate	The intermediate converts into the product within 30 minutes by stirring at 82°C
		46 %, + 4% intermediate	The intermediate converts into the product within 30 minutes by stirring at 82°C
		22 %	contains some by-products
		5 %, + 3 % of [Tc(CO)$_3$ (dien)]	relatively low yield probably due to adsorption of the hydrophobic product to the solid phase material.

CLAIMS

1. Process for generating a water soluble metal complexed agent, comprising contacting (I) a solid phase bound organic conjugate represented by the formula



(I)

wherein:

C is a methylene group that may be substituted by one or more electron withdrawing groups, in particular RO, RS or R N, wherein R is an aliphatic or arylic group

L is a linker that may or may not be present, that is coupled to the solid support and has activating properties towards nucleophilic attack to the C group and is preferably a phenyl, allyl or aryl; and

R1 and R2 are the same or different metal coordinating group, which solid phase bound organic conjugate is derivatized at one or both of R1 and R2 with a biologically active molecule,

with (II) $[M(H_2O)_3(CO)_3]$,

wherein M is selected from the group consisting of technetium (Tc), rhenium (Re), ruthenium (Ru), rhodium (Rh), platinum (Pt), iridium (Ir) and copper (Cu);

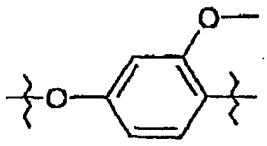
under suitable conditions to cause the formation of a coordinate bond between $[M(H_2O)_3(CO)_3]$ and the tertiary amine nitrogen atom of the solid phase bound organic conjugate and thereby the release of the metal complexed agent thus formed

from the support.

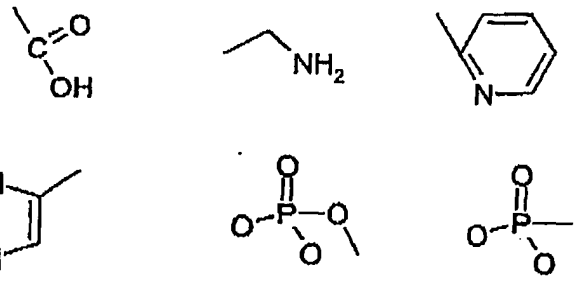
2. Process according to claim 1, wherein the linker is selected from the group consisting of phenyl, vinyl, aryl.

3. Process according to claim 2, wherein the phenyl, vinyl, aryl are substituted with OR, R, NR_2, wherein R is an aliphatic or arylic group.

4. Process according to claims 2 and 3, wherein the linker is a group of the formula



5. Process according to any one of the claims 1-4, wherein R1 and R2 are selected from the group consisting of



6. Process according to any one of the claims 1-5, wherein M is selected from the group consisting of Tc, Re, Ru, Rh, Ir, Cu and Pt.

7. Process as claimed in claim 6, wherein the metal is selected from the group consisting of ^{99m}Tc, ^{186}Re and ^{188}Re.

8. Process according to any one of the claims 1-7, wherein the biomolecule is selected from the group consisting of amino acids; steroids; peptides; proteins, in particular

structural proteins, enzymes or antibodies; carbohydrates; polysaccharides, oligosaccharides; nucleosides; nucleotides; oligonucleotides, polynucleotides; lipids, peptides.

9. Process according to any one of the claims 1-8, wherein the solid support is a polyethylene glycol resin, or a hybrid of polyethylene glycol and polystyrene, e.g. a polystyrene resin with polyethylene glycol spacers with a benzyl alcohol anchoring group.

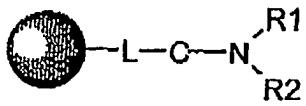
10. Process according to any one of the claims 1-9, further comprising the step of collecting the metal complexed agent for further use.

11. Process according to any one of the claims 1-10, wherein the process is performed at a pH that is in the range of about 6.0-11.0, preferably in the range of about 7.5-9.5.

12. Process according to any one of the claims 1-11, wherein the process is performed at a temperature in the range of about 40-100°C, preferably in the range of about 70-82°C.

13. Process according to any of the claims 10-12, which further comprises bringing the collected metal labeled conjugate into a pharmaceutically acceptable form.

14. A solid phase bound organic conjugate represented by the formula



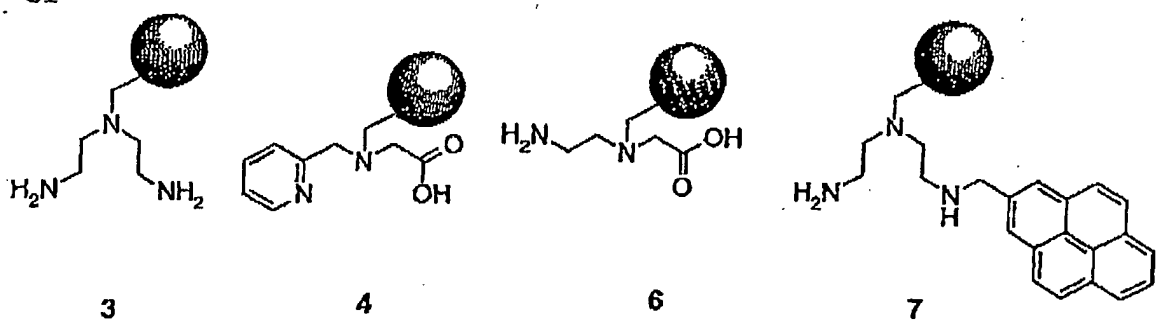
(I)

wherein L, C, R1 and R2 are as defined in claims 1-7.

15. A solid phase bound organic molecule according to claim 14, characterized in that the biologically active molecule is selected from the group consisting of amino acids; steroids; peptides; proteins, in particular structural proteins, enzymes or antibodies; carbohydrates; polysaccharides, oligosaccharides; nucleosides; nucleotides; oligonucleotides, polynucleotides; lipids, peptides.

16. A solid phase bound organic molecule according to any one of the claims 14-15, wherein the solid support is a polyethylene glycol resin, or a hybrid of polyethylene glycol and polystyrene, e.g. a polystyrene resin with polyethylene glycol spacers with a benzyl alcohol anchoring group.

17. A solid phase bound organic molecule according to any one of the claims 14-16, selected from the group consisting of



3 4 6 7

18. A metal complexed organic molecule obtainable by the process according to any one of the claims 1-13.

19. A kit for the preparation of a diagnostic or therapeutic pharmaceutical composition, comprising a container with the molecule of formula (I), in which the reaction with a solution of $[M(H_2O)_3(CO)_3]$ can take place.

20. Kit as claimed in claim 21, wherein the container is a vessel or column.

21. Kit as claimed in any one of claims 19 and 20,

further comprising a solution of $[M(H_2O)_3(CO)_3]$.

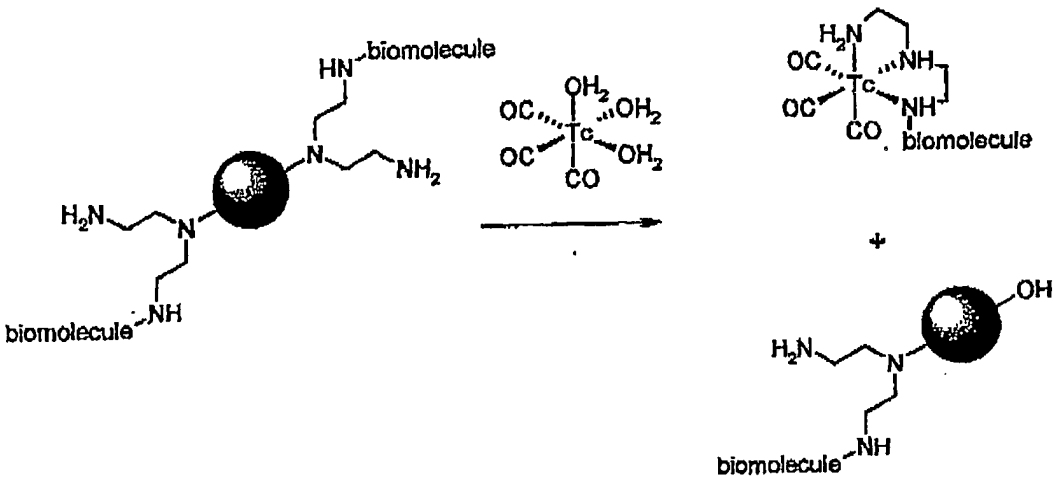
22. Kit as claimed in any one of claims 19 and 20, further comprising the reagents for the preparation of the metal carbonyl $[M(H_2O)_3(CO)_3]$.

23. Kit as claimed in any one of the claims 19-22, further comprising a facility for filtration.

ABSTRACT

The present invention relates to Process for generating a water soluble metal complexed agent, comprising contacting a solid phase bound organic conjugate represented by the formula I with $[M(H_2O)_3(CO)_3]$, under suitable conditions to cause the formation of a coordinate bond between $[M(H_2O)_3(CO)_3]$ and the tertiary amine nitrogen atom of the solid phase bound organic conjugate and thereby the release of the metal complexed agent thus formed from the support. The invention further relates to the conjugate of formula I and to a kit for performing the process.

Figure 1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biomolecule
N
NH2
H2N
N
biomolecule
NH
OH2
OH2
OC
Tc
OC
OH2
CO
H2N
NH
OC
Tc
OC
NH
CO
biomolecule
+
OH
H2N
N
biomolecule
NH

Figure 2

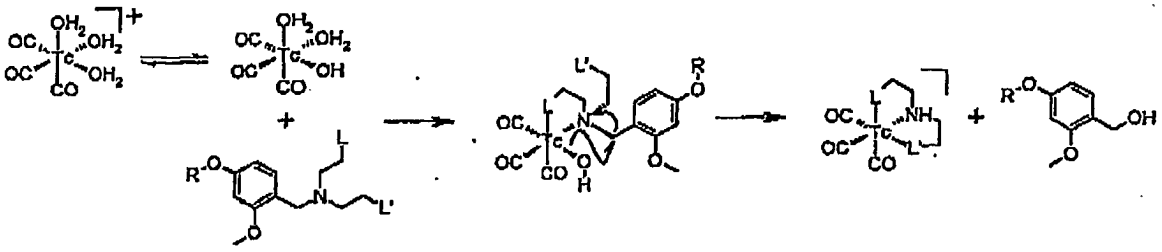


Figure 3

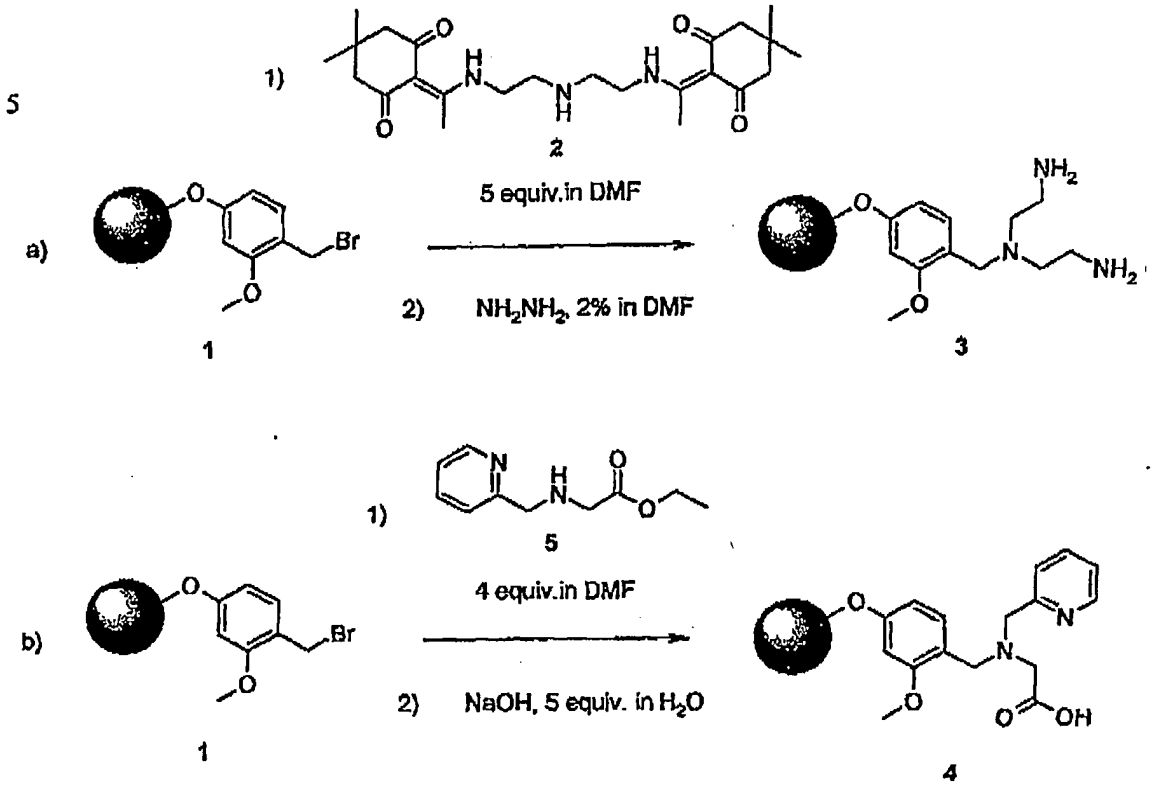

1)
2
5 equiv.in DMF
a)
Br
2) NH_2NH_2, 2% in DMF
1
3
NH_2
NH_2
1)
5
4 equiv.in DMF
b)
Br
2) NaOH, 5 equiv. in H_2O
1
4
OH

Figure 4

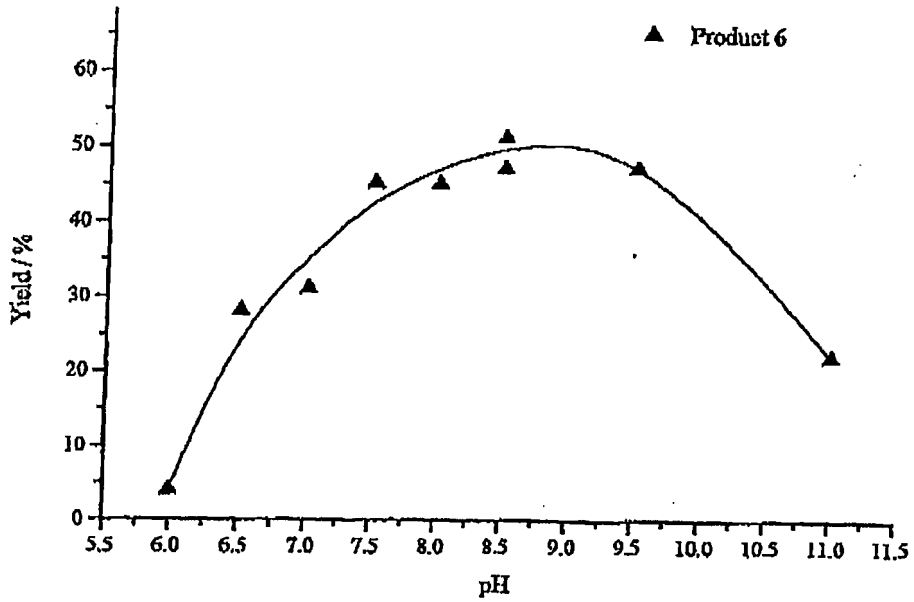
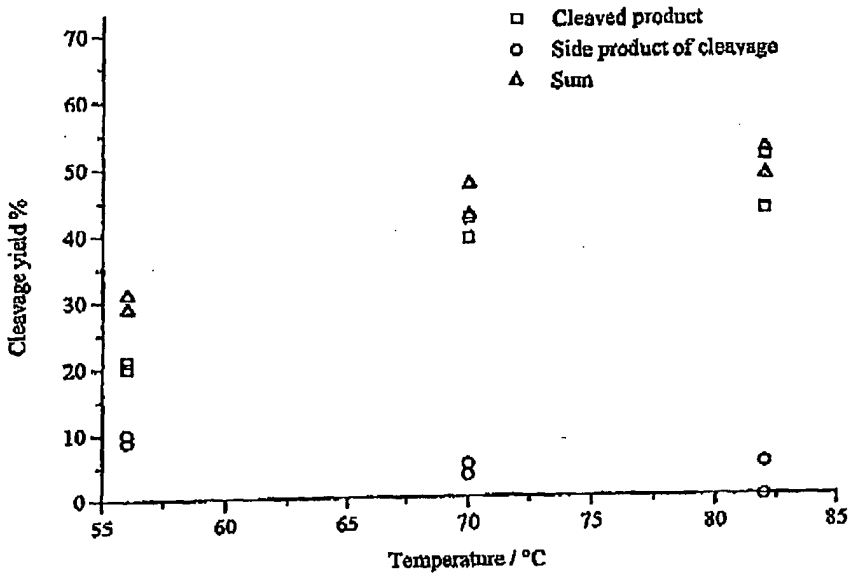

Product 6
Yield/%
pH
0
10
20
30
40
50
60
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5

Figure 5


Cleaved product
Side product of cleavage
Sum
Cleavage yield %
Temperature / °C
0
10
20
30
40
50
60
70
55
60
65
70
75
80
85